



MICROSCOOP[®] / MINT

Spatial Proteomics with MICROSCOOP[®]

Instructions for use of Synlight-Rich[™] Kit

The reagent kits for sample preparation and photolabeling under Microscoop[®].

Catalog Number : SYN-RI0206 (Up to 6 reactions)

December 2024

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1. INTENDED USE

The Microscop[®] system performs automated ultra-content microscopy-guided photo-biotinylation, enabling the photolabeling and isolation of subcellular proteins for mass spectrometry-based proteomic discovery. The Synlight-Rich[™] Kit is for sample preparation and photolabeling under Microscop[®] system. All reagents in Synlight-Rich[™] Kit are for research use only (RUO).

2. INTRODUCTION

The Microscop[®] system is a powerful platform for spatial proteomics exploration. Syncell integrates microscopy, optics, mechatronic, photochemistry, and deep learning or traditional image processing to enable high-content *in situ* photolabeling. The Microscop[®] system photolabels proteins at user defined regions of interests (ROIs) under a microscope utilizing directed photochemistry. Photolabeled proteins are then extracted and sent for mass spectrometry analysis to reveal known and novel protein players located at user-specified ROIs. Thus, beyond spatial proteomics mapping, subcellular spatial proteomics discovery can be achieved.

The Synlight-Rich[™] Kits have been meticulously developed to be used with the Microscop[®] system. These reagents in the kit enable high efficiency photolabeling for sub-micron or micron size structures. The Synlight-Rich[™] Kits provide comprehensive instructions and all essential materials for the systematic preparation and photolabeling of samples cell and tissue. The Synlight-Rich[™] Kit contains materials sufficient for six reactions; catalog number SYN-RI0106 includes a positive control reagent for photolabeling efficiency checking and catalog number SYN-RI0206 has no positive control. Once cells or tissues have been prepared with reagents from the Synlight-Rich[™] Kit, cells or tissues are ready for Synpull[™] Kit (Catalog Number: SYN-PU0106). Multi sample slides are scraped and combined to increase the total protein contents to fit the sensitivity of mass spectrometry. The scraped sample is then lysed, enriched, and digested into peptides before being sent to a mass spectrometer to perform LC-MS/MS analysis.

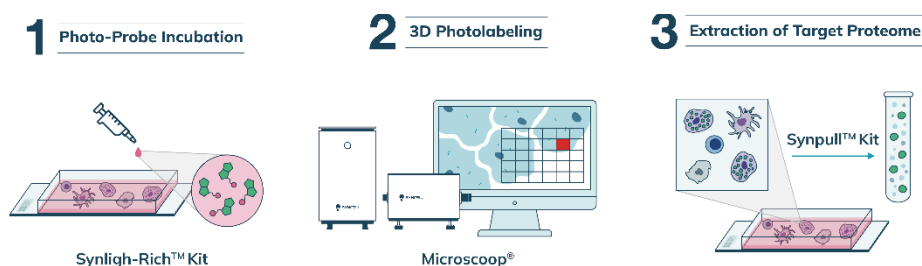


Fig. 1.1 Workflow of De Novo Spatial Proteomics

1. Samples are immersed in the photoactivable probe of the Synlight-Rich[™] Kit.
2. Three-dimensional photolabeling of regions of interest (ROI) with Microscop[®].
3. Labeled proteins are extracted using the Synpull[™] Kit for LC-MS/MS analysis

3. KIT COMPONENTS AND REAGENTS

3.1 Catalog number SYN-RI0206 (Up to 6 reactions)

Component	Quantity	Dilution Factor	Reagent
Block 1	120 μ L	50 \times	Block sample , Block 1 substance in water
Block 2	120 μ L	50 \times	Block sample , Block 2 substance in dimethyl sulfoxide (DMSO)
Photolabel	120 μ L	50 \times	Photolabeling reagent, Photo-activatable probe in DMSO
Quench	360 μ L	50 \times	Quench photolabeling reaction, Quench mixture in water

3.2 Warnings and Precautions

- For research use only.
- Do not use the kit contents beyond the expiration date.
- If the reagent solution contacts the skin or eye, flush with copious amounts of water.
- Dispose of containers and unused contents in accordance with Federal, State and Local regulatory requirements.
- Wear suitable protective clothing, gloves, and eye/face protection when handling the contents of this kit.
- Wash hands thoroughly after handling.

4. MATERIALS REQUIRED BUT NOT PROVIDED

4.1 Consumables

Item	Description
Single-well slide	Thickness no. 1.5 high-performance cover glass (0.170 ± 0.005 mm), e.g., P/N C1-1.5 H-N, Cellvis
Adhesion microscope slide	For tissue applications, e.g., EpreDia™ SuperFrost Plus™ Adhesion slides
Microscope cover glasses	For tissue applications; thickness no. 1 or 1.5 (0.13–0.19 mm)
Double-sided tape	For tissue applications, approximately 2 mm wide, e.g., iSpacer® double sided sticky (cat. IS201/IS211, SUNJin Lab)
Triton™ X-100	Laboratory grade; for dilution and washing buffers

	Alternative reagent: Tergitol™ 15-S-9, NP-40, or Tween® 20
ROI fluorescent dye	Secondary antibody with fluorescent dye excited by light > 470 nm wavelength, e.g., Alexa Fluor™ 488, Alexa Fluor™ 555, Alexa Fluor™ 568, and Alexa Fluor™ 647
DNA-staining dye	Cell-permeable DNA-staining dye that is excited by ultraviolet light and emits blue fluorescence at 460–490 nm, e.g., Hoechst 33342

4.2 Equipment

Item	Description
Inverted confocal microscope	For checking photolabeled protein signals through 3D confocal imaging, e.g., ZEISS LSM880
Light-controlled environment	Lighting at a wavelength of >500 nm to prevent undesirable reactions with the photolabeling reagent
Laser safety goggles	Designed to shield eyes from laser wavelengths (750–850 nm), e.g., Thorlabs LG12 laser safety glasses with amber lenses (11% visible light transmission)

4.3 Precautions

- Cells require prior permeabilization (see SAMPLE PREPERATION Section 6.1).
- Before treating sample with Block 1 Buffer and Block 2 Buffer, sample can be blocked with general blocking buffer, e.g., 3% bovine serum albumin (BSA) and 0.1% Triton™ X-100 phosphate-buffered saline (PBS) .
- Opening the Photolabel tubes and operating the photolabeling procedures should be performed in a light-controlled environment. Ensure the lighting is at a wavelength of > 500 nm to prevent undesirable reactions.
- The wavelength for photolabeling efficiency checking with Verify is near 490nm. If your ROI's fluorescence signal falls within the wavelength range of 470–520 nm (e.g., Alexa Fluor™ 488), please contact the SYNCCELL team by email: Info@syncell.com.

5. STORAGE

The Synlight-Rich™ Kits should be stored at 2–8 °C upon receipt. Under these conditions, the kit components will remain stable until the expiration date indicated on the outer box.

6. SAMPLE PREPARATION

6.1 Cells

Use cells that can adhere to a single-well slide after being fixed with appropriate fixation buffers.

To achieve optimal imaging of ROIs, it is essential to experiment with the cell seeding density. As a general guideline, start with a seeding density of 2×10^5 to 4×10^5 cells per well. If ROI targets are at the cell edge or synapse, seeding density can be 0.8×10^5 .

6.2 Tissue Sections

Either formalin-fixed, paraffin-embedded (FFPE) tissue sections or frozen (OCT-embedded) tissue sections can be used, with a thickness up to 20 μm .

For FFPE tissue sections, prior antigen retrieval is required. This is necessary to reverse the cross-links formed by formalin and to unmask the epitopes, making them accessible to the antibodies.

6.3 ROI identification for Microscoop®

Use cell/tissue samples that can be visualized under a fluorescence microscope to identify ROIs. Any interesting target in cell and tissue can be identified by using immunostaining or by detecting endogenous expression at imaging wavelength above 470 nm.

6.4 Sample Number

The suggested starting materials for different target sizes are listed in the table below, and it is recommended to prepare unlabeled (UL) and photolabeled (PL) samples in pairs.

Category	Cell line / tissue type	Target and size (top view)	No. of slides	No. of cells / area per slide
Cell	U-2 OS	Nucleus ($\sim 20 \mu\text{m}^2$)	2 (UL); 2 (PL)	2.4×10^5
	U-2 OS	Mitochondrion ($\sim 0.8\text{--}3.0 \mu\text{m}^2$)	3 (UL); 3 (PL)	2.4×10^5
	RPE-1	Cilium ($\sim 0.2\text{--}0.7 \mu\text{m}^2$)	6 (UL); 6 (PL)	2.0×10^5
Tissue	Mouse brain	Nucleus ($\sim 20 \mu\text{m}^2$)	3 (UL); 3 (PL)	300 mm^2

7. REAGENT PREPARATION

7.1 Dilution and Washing Buffer

Prepare phosphate-buffered saline with Triton™ X-100 (PBSTx) for the dilution and washing steps:

0.1% PBSTx for Cell Samples: Add 1 mL of 10% Triton™ X-100 to 99 mL of PBS.

0.5% PBSTx for Tissue Samples: Add 5 mL of 10% Triton™ X-100 to 95 mL of PBS.

7.2 Blocking Buffer

Block 1 Buffer: Retrieve Block 1 (A), vortex at 500 rpm for 5 min at room temperature (RT), and spin down briefly. For each pair of samples (1 PL, 1UL), freshly prepare Block 1 Buffer by mixing 40 µL of Block 1 (A) with 1960 µL 0.1% PBSTx (for cells)/ 0.5% PBSTx (for tissues).

Block 2 Buffer: Retrieve Block 2 (B), vortex at 500 rpm for 5 min at room temperature (RT), and spin down briefly. For each pair of samples, freshly prepare Block 2 Buffer by mixing 40 µL of Block 2 (B) with 1960 µL 0.1% PBSTx (for cells)/ 0.5% PBSTx (for tissues) for a paired sample.

7.3 Labeling Reagent and Quencher

Labeling Reagent: Retrieve Photolabel (C), vortex at 500 rpm for 10 min at RT, and then spin down briefly. Freshly prepare adequate Labeling Reagent by diluting the Photolabel for 50-fold using PBS. For each pair of samples, mix 40 µL of Photolabel (C) with 1960 µL of PBS for paired cell samples. For a large target such as the nucleus, the Photolabel (C) can be diluted for 100-fold as the Labeling Reagent.

Quencher: Retrieve Quench (D), vortex at 500 rpm for 5 min at RT, then spin down briefly. For each pair of samples, freshly prepare Quencher by mixing 120 µL of Quench (D) with 5.88 mL of 0.1% PBSTx (for cells)/0.5% PBSTx (for tissues).

7.4 Verification Buffer

Verification Buffer: Vortex Verify (G) in positive control Synlight-Rich™ Kit (catalog number SYN-RI0106) or Verify (P) in Synpull™ Kit, then spin down briefly. Freshly prepare the Verification Buffer by mixing 20 µL of Verify with 980 µL of 3% BSA/0.1% PBSTx for 1 PL samples. For tissue samples, prepare the Verification Buffer by performing a 1:50 dilution of Verify with 0.5% PBSTx.

8. TEST PROCEDURES

8.1 Blocking

Step1: Add 1 mL of Block 1 Buffer to the UL and PL samples, respectively. Incubate for 30 min (for cells) or 60 min (for tissues) at RT.

Step2: Discard the Block 1 Buffer. Rinse the samples three times with 0.1% PBSTx (for cells) or 0.5% PBSTx (for tissues).

Step3: Add 1 mL of Block 2 Buffer to the UL and PL samples, respectively. Incubate for 15 min (for cells) or 60 min (for tissues) at RT.

Step4: Discard the Block 2 Buffer. Rinse the samples three times with 0.1% PBSTx (for cells) or 0.5% PBSTx (for tissues).

Note 1: This is a stop point. After this step, the samples can be stored at 2–8°C for up to one week.

8.2 Staining ROI marker

Step1: Stain the UL and PL samples using the ROI marker (primary antibody) under optimal conditions.

Step2: Wash the samples with 0.1% PBSTx (for cells) or 0.5% PBSTx (for tissues) three times before placing them on a seesaw shaker for 10 min (for cells) or 30 min (for tissues) per wash.

Step3: Stain samples with the ROI fluorescent dye:

Stain the ROIs with a fluorescent dye > 470 nm wavelength (e.g., a secondary antibody labeled with Alexa Fluor™ 568) under optimized conditions.

Step4: Wash the samples with 0.1% PBSTx (for cells)/0.5% PBSTx (for tissues) three times, placing them on a seesaw shaker for 10 min per wash.

Step5: For samples with intrinsic fluorescence > 470 nm, skip Steps 1 through 4.

Step6: Rinse the samples three times with PBS.

8.3 Photolabeling (Under Light-controlled Environment)

Step1: Addition of Labeling Reagent to the paired samples:

For cell samples: Add 1 mL of **Labeling Reagent** to each single-well slide.

For tissue samples:

- Affix 0.05 mm thick double-sided tape (iSpacer®, cat. IS201/IS211, SUNJin Lab) around the tissue perimeter to form a rectangle.
- Cover the tissue sample with an adequate amount of Labeling Reagent (typically 100–200 µL).
- Overlay a microscope cover glass on the tissue.
- Secure the cover glass by sealing the perimeter with double-sided tape.

Step2: Operate Microscop® in accordance with the user manual to photolabel the designated ROIs.

Caution: DO NOT use DAPI (365 nm) or brightfield imaging when samples are immersed in the Labeling Reagent.

Note 2: Place UL sample on the stage of the microscope when photolabeling PL sample to ensure both samples undergo similar process (e.g., temperature and immersion time) with exception for the photolabeling step.

Note 3: For photolabeling of tissue sections, place the microslide upside down (i.e. tissue sections facing the objective) on the stage of the microscope to photolabel the designated ROIs.

Step3: Discard the Labeling Reagent and wash the samples:

For cell samples:

- Discard the Labeling Reagent.
- Wash the samples three times with 1 mL of Quencher, placing them on a seesaw shaker for 5 min per wash.

For tissue samples:

- Carefully remove cover glass and double-sided tape.
- Discard the Labeling Reagent.
- Incubate the samples with 1 mL of Quencher for 5 min and then remove. Repeat this step twice.

Step4: Rinse the samples with PBS and store at 2–8°C.

8.4 Photolabeling Efficiency Measurement

Caution: Samples applied with Verification Buffer are unsuitable for proteomic treatment. Thus, during the scraping procedure, save most of the sample for further proteomic treatment, leaving only a small area of cells to measure photolabeling efficiency using the Verification Buffer.

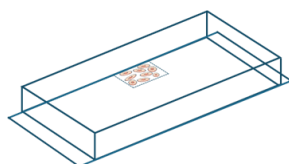


Fig. 8.1 Photolabeled cells may be retained near the well edge during the scraping procedure and used to measure the photolabeling efficiency and accuracy.

Step1: Addition of Verification Buffer to the PL samples.

For cell samples: Add 1 mL of **Verification Buffer** to the PL samples.

For tissues on a glass slide: Apply 100–200 μL of **Verification Buffer** to the PL tissue samples. Place coverslips over the samples to prevent them from drying out.

Step2: Incubate the samples for 1 hr (for cells) or 3 hrs (for tissues) in the dark at RT on a seesaw shaker.

Step3: Wash the samples with 0.1% PBSTx (for cells) or 0.5% PBSTx (for tissues) three times, placing them on a seesaw shaker for 5 min per wash.

Step4: Rinse the samples with PBS three times.

Step5: The samples are now ready for measurement. Use Microscoop[®] with the FITC channel (490nm) for epifluorescence imaging.

Parameters for epifluorescence imaging by Microscoop[®] :

Sample Type	Objective	Resolution	Channel	Lamp Intensity	Exposure Time
Cell	Plan Apo Lmbd 10.0x/0.45/Dry (PFS)	800 x 800	FITC (490nm)	10 % (~ 1mw)	1000– 2000 ms
Cell	Plan Apo LmbdD 40.0x/0.95/Dry (PFS)	800 x 800	FITC (490nm)	5 % (~ 0.4mw)	400–600 ms
Tissue	Plan Apo LmbdD 40.0x/0.95/Dry (PFS)	800 x 800	FITC (490nm)	10 % (~ 1mw)	500–1000 ms

Labeling efficiency can be quantified by the signal-to-noise ratio (S/N ratio) derived from the epifluorescence imaging:

$$S/N \text{ ratio} = \frac{\text{Intensity of ROIs in PL} - \text{Blank}}{\text{Intensity of unlabeled area within cell in PL} - \text{Blank}}$$

where *Intensity* is the mean fluorescence intensity of the imaging channel;

Blank is the mean fluorescence intensity of the imaging channel in the areas without cells.

Analyze labeling efficiency (**S/N ratio**) using ImageJ:

Step5-1: Drag the image of PL samples already stained with Verification Buffer into ImageJ, then select “Image > Adjust > Brightness/Contrast” to tweak the image for the optimal visualization of the ROIs (nuclei).

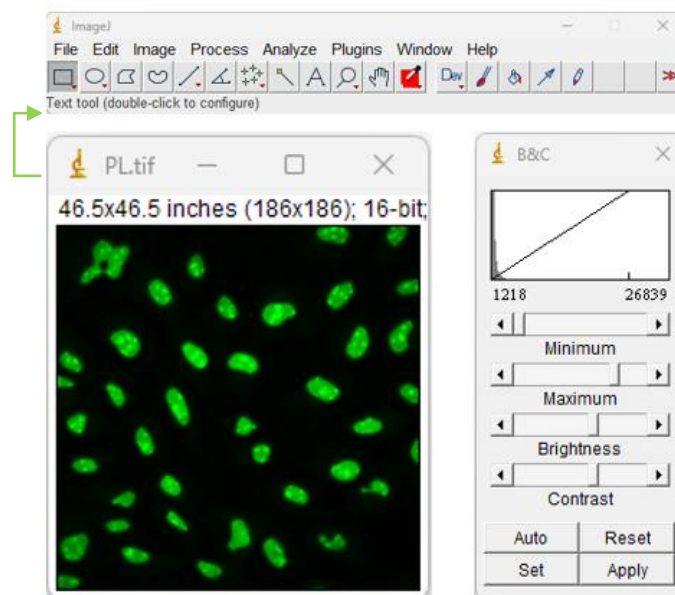


Fig. 8.2 The optimal visualization of the ROIs (nuclei)

Step5-2: To determine the ROIs, click “Image > Adjust > Threshold.” Drag the lower scrollbar to the maximum value and upper scrollbar to a position at which the ROIs (nuclei) are properly filled with red color.

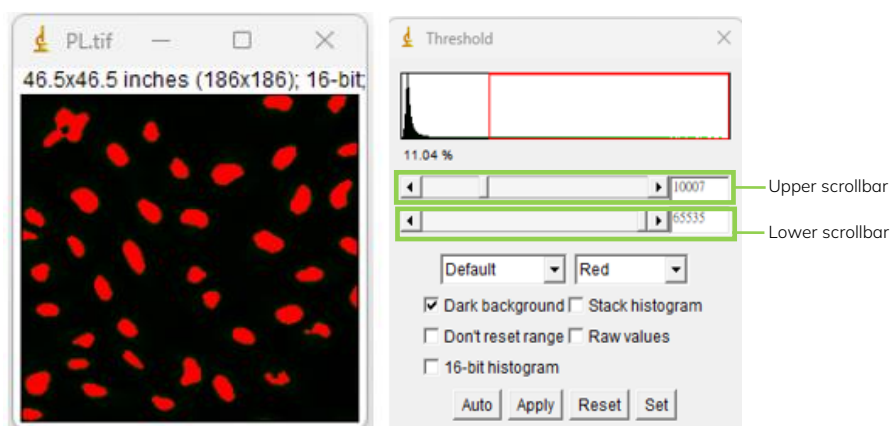


Fig. 8.3 Selected labeled ROIs

Step5-3: To measure the mean intensity of labeled ROIs (nuclei), click “Analyze > Measure”

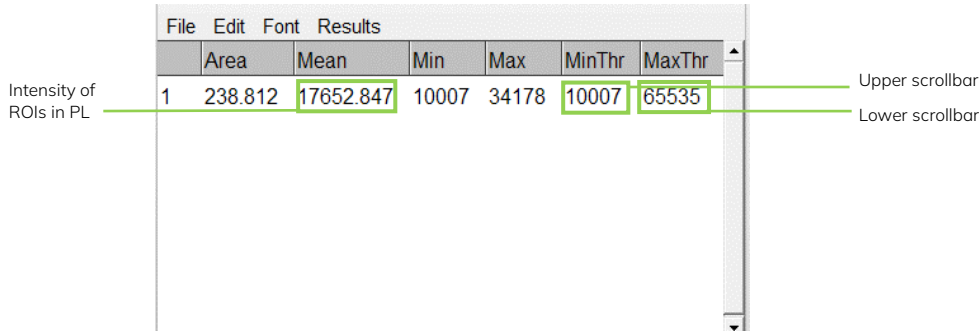


Fig. 8.4 The mean intensity of labeled ROIs (nuclei)

Step5-4: To determine the Blank, click “Image > Adjust > Threshold.” Drag the upper scrollbar to 0; then, drag the lower scrollbar to a position at which the areas without cells are properly filled with red color, which is usually at “the peak of the histogram.”

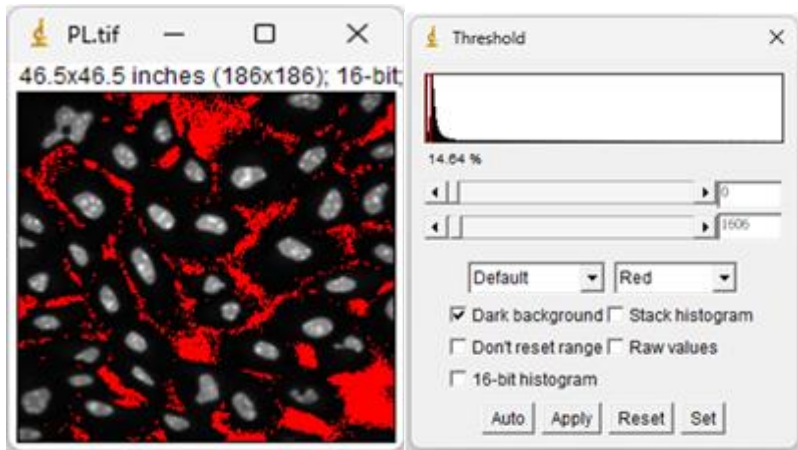


Fig. 8.5 Selected Blank area

Step5-5: To measure the mean intensity of Blank, click “Analyze > Measure.”

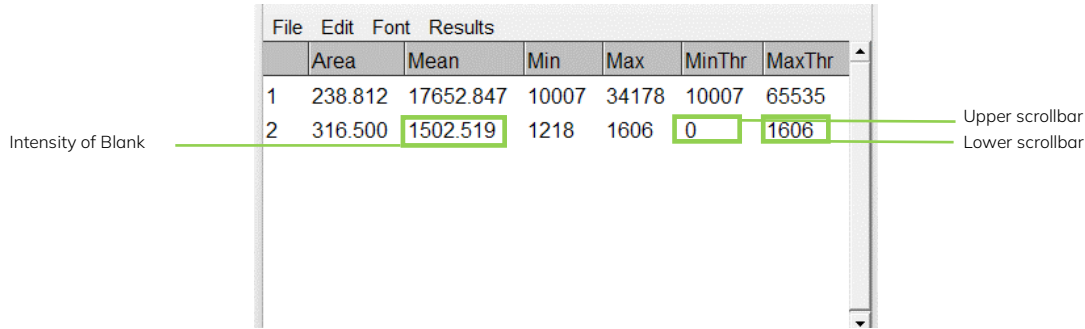


Fig. 8.6 The mean intensity of Blank

Step5-6: To measure the mean intensity of unlabeled area within cell, click “Image > Adjust > Threshold.” Drag the scrollbar between Blank and labeled ROI. The upper scrollbar is near MaxThr of Blank, and the lower scrollbar is near MinThr of labeled ROI.

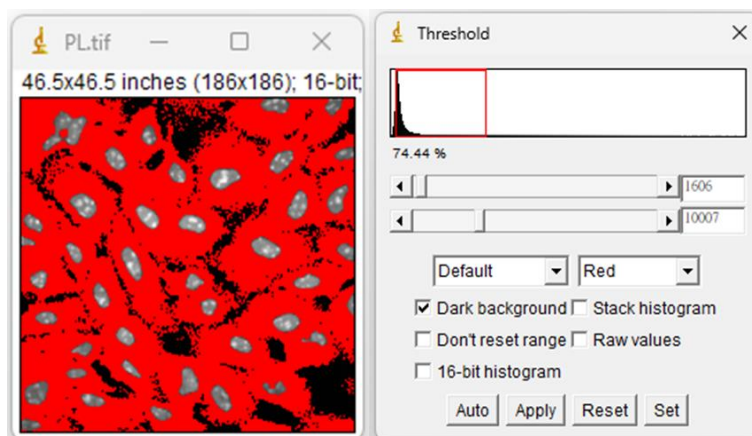


Fig. 8.7 Selected unlabeled area within cell

Step5-7: To measure the mean intensity of without labeling cell, click “Analyze > Measure.”

	Area	Mean	Min	Max	MinThr	MaxThr
1	238.812	17652.847	10007	34178	10007	65535
2	316.500	1502.519	1218	1606	0	1606
3	1609.500	2318.003	1606	10007	1606	10007

Fig. 8.8 The mean intensity of unlabeled area within cell

Step5-8: Calculate the photolabeling efficiency using the measured intensities:

$$\begin{aligned}
 \text{S/N ratio} &= \frac{\text{Intensity of ROIs in PL} - \text{Blank}}{\text{Intensity of unlabeled area within cell} - \text{Blank}} \\
 &= \frac{17652.847 - 1502.519}{2318.003 - 1502.519} = 19.805
 \end{aligned}$$

Step6: check labeling accuracy, use an inverted confocal microscopy system with the imaging channel of excitation at 493 nm and emission at 518 nm.

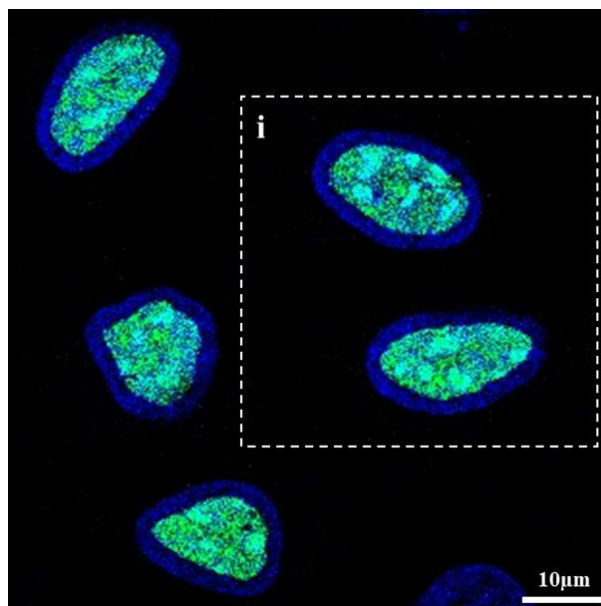


Fig. 8.9 The confocal fluorescence image of a photolabeled PL sample

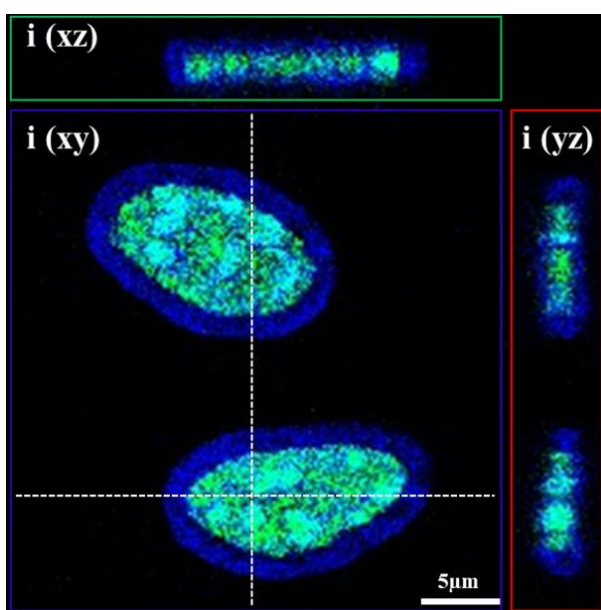


Fig. 8.10 The orthogonal view of confocal image

The photolabeling signal of PL samples, visualized with Verification Buffer using the FITC channel, is shown in green. The signal is localized in the cell nuclei, which is stained with Hoechst 33342 (Thermo Scientific) and shown in blue via the 405 nm channel. The confocal images demonstrate accurate labeling of the nuclei on both the xy- and z- axes (imaging by ZEISS LSM880; 63× oil objective lens).

8.5 Suggested Acceptance Criteria for Photolabeled Samples

- I. Labeling accuracy: the labeling signal should be localized in the target.
- II. Signal-to-noise ratio (S/N ratio) should be greater than 8.0.

Note 4: A 2.4×10^5 cell nuclei sample with an average S/N ratio at 8.0 processed with Synpull™ Kit in combination with the Orbitrap Fusion™ Lumos™ MS typically yields at least 500 total protein identifications. When the targeted structures are particularly small or sparse, it is necessary to use more cells/tissues or achieve a higher S/N ratio to obtain optimal results.

To improve labeling efficiency, consider the followings:

- a. Increase the concentration of the labeling reagent.
- b. Increase the power setting.
- c. Increase the labeling exposure time.

Caution: Excessive laser power or prolonged labeling exposure time can damage the target structures. Please keep the laser power below 200 mW and the labeling exposure time below 1000 μ s.

8.6 Summary of Synlight-Rich™ Kit workflow

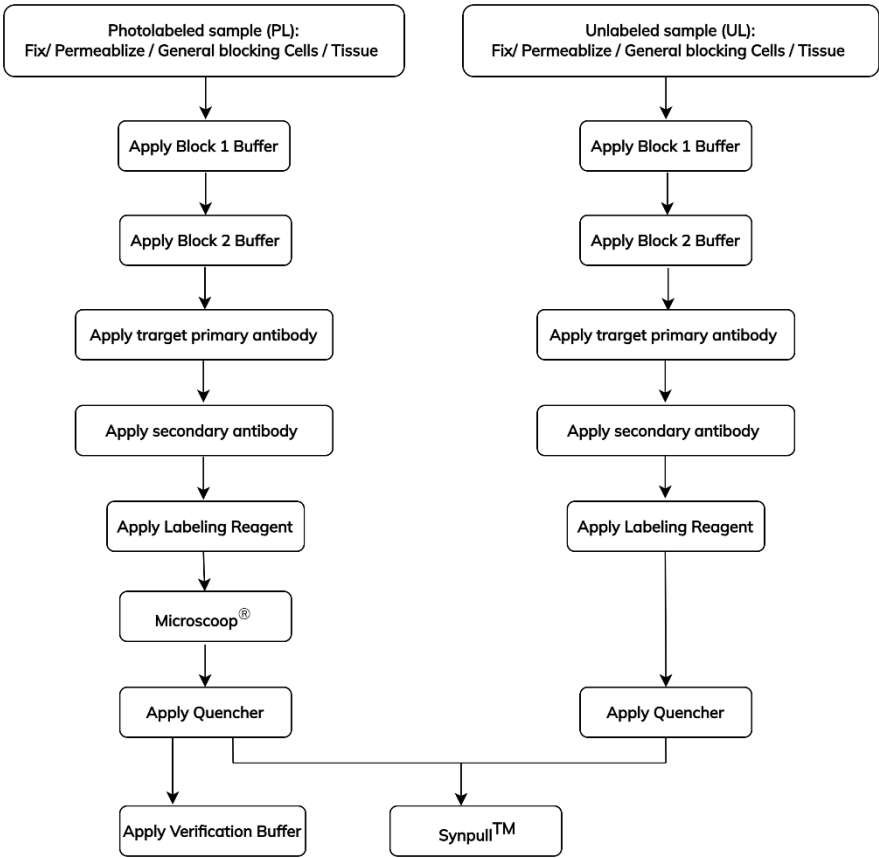


Fig. 8.11 The flow chart of Synlight-Rich™ Kit.

9. PURCHASER NOTIFICATION

9.1 Contact information

Manufacturer: SYNCCELL (TAIWAN) Inc.

Email: Info@syncell.com

Tel: +886-2-2785-6780

Address: 14F, No. 508, Sec. 7, Zhongxiao E. Rd., Nangang Dist., Taipei City 115, Taiwan

European Authorized Representative: Luana Med B.V.

Address: Abtswoudseweg 18, 2627AL, Delft, NL.

9.2 Customer support

For the latest services and support information for all locations, go to

<https://www.syncell.com/>.

On the website, you can

search through frequently asked questions (FAQs)













submit a question directly to Technical Support

Safety data sheets (SDSs) are available at the QR code below:



For research use only; not for use in diagnostic procedures

9.3 Symbols

 REF	Catalog number		Temperature limit
 LOT	Batch code		Indicates the total number of tests that can be performed with the product
	Date of manufacture		Consult instructions for use
	Use by date		Storage condition (avoid light): keep away from sunlight
	Manufacturer		Positive control
	CE marking		Authorized representative in the European Community/ European Union